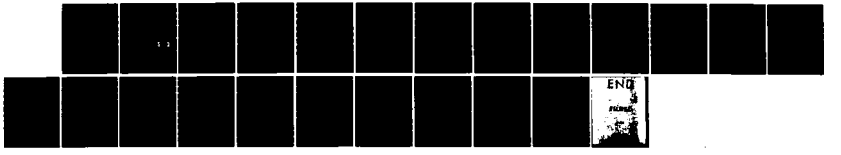
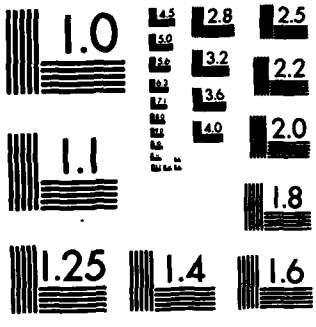
AD-A144 101 MECHANISMS OF RAPID NONSPECIFIC RESISTANCE INDUCED BY IMMUNOMODULATORS: D..(U) MEDICAL COLL OF PENNSYLVANIA PHILADELPHIA DEPT OF MICROBIOLOG.. P S MORAHAN ET AL. 1/1

UNCLASSIFIED 30 JUL 84 N00014-82-K-0669 F/G 6/5 NL





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REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: 1	2. GOVT ACCESSION NO.: AD-A144 101
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): Mechanisms of Rapid Nonspecific Resistence Induced by Immunomodulators: Delineation Using Selective Depletion of Cells	5. TYPE OF REPORT & PERIOD COVERED: 7/1/83 - 6/30/84
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Page S. Morahan, Ph.D. Alvin Volkman, M.D.	8. CONTRACT OR GRANT NUMBER(s): N00014-82-K-0669
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Dept. Microbiology & Immunology, 3300 Henry Ave., Medical College Penn., Philadelphia, PA 19129 and Dept. Pathology, E.C.U.M.S., Greenville, NC 27834	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 666-008
11. CONTROLLING OFFICE NAME AND ADDRESS: Jeannine A. Maide, Ph.D., Scientific Officer Immunology Code 441, Cellular Biosystems Group, Dept. of the Navy, ONR, Arlington, VA 22217	12. REPORT DATE: 7/30/84 13. NUMBER OF PAGES: 21
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): Same as 11	15. SECURITY CLASS. (of this report): Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report): Unlimited	
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report): Unlimited	
18. SUPPLEMENTARY NOTES	

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Macrophages, monocytes, PMN, NKcells, herpes simplex type 2, encephalomyocarditis virus, Listeria, host defense, immunotherapy, interferon, immunomodulators, C. parvum, MVE-2, Muramyl dipeptide, lipoidal amines, ^{89}Sr, bone marrow depletion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have analyzed the effects of ^{89}Sr treatment on various effector cell populations, including circulating monocytes and PMN, resident peritoneal MØ, and splenic NK and NC cells. The short term ^{89}Sr regimen is now sufficiently characterized to be useful in predicting which nonspecific effector cell populations are important in natural and immunomodulator-enhanced host resistance. Within a week after ^{89}Sr treatment, there is a profound decrease in the numbers of all circulating white blood cells, no marked change in resident peritoneal MØ, a significant decrease in spontaneous as well as interferon-inducible NK-

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PROGRESS REPORT -- [redacted]

1.0 Experimental objectives. During the first two years our objectives have been to:

1.1 Establish the kinetics of the effects of short term and long term treatment with ^{89}Sr on nonspecific effector cells in the CD1 female mouse.

1.2 Establish the efficacy of selected immunomodulators on resistance to Listeria monocytogenes, EMC virus and HSV-2 in normal mice and in mice treated with the two ^{89}Sr regimens.

2.0 Detailed progress report

2.1 Kinetics of the effects of short term and long term ^{89}Sr on various effector cell populations. Three complete experiments and parts of others have been directed at defining the kinetics of the ^{89}Sr effects on circulating monocytes, lymphocytes and PMN, peritoneal MØ, and splenic NK and NC cells. Detailed information has been generated on the effects of acute and prolonged bone marrow failure on these cells, and on the kinetics of restoration (if any) following establishment of extra-medullary hematopoiesis.

Fortuitously, the CD1 mouse has turned out to be a mouse that has low spontaneous NK cell activity. Within a week after administration of 4 uCi/gbw of ^{89}Sr, there is a profound decrease in the numbers of all circulating white blood cells, no marked changes in peritoneal MØ and lymphocytes, a significant decrease in spontaneous as well as interferon-inducible NK cell activity, and no effect on splenic NC cell activity (Figs. 1-3). PMN exudation appears normal, while monocyte elicitation into an inflammatory focus in the peritoneal cavity is virtually eliminated. However, the resident peri- toneal MØ is able to be activated in situ by C. parvum or thioglycollate broth. Thus, in the early period after ^{89}Sr in the CD1 mouse, there is a marked decrease in the functions of both monocytes and NK cells, with the maintenance of an intact compartment of tissue macrophages.

We are still defining the long term effects after one and two treatments with ^{89}Sr. Our present major conclusions are: (i) although circulating monocytes remain below normal, the ability to elicit monocytes into an inflammatory focus returns after about 35-50 days (Table 1); (ii) the number of circulating monocytes, lymphocytes and PMN were relatively depressed at most times examined after ^{89}Sr (Figure 1); (iii) spontaneous splenic NK cell activity is low for at least 35 days, and begins to return towards normal levels unless there is a second ^{89}Sr treatment. However, ^{89}Sr completely eliminates the ability of splenic NK cells to be activated by interferon (Figure 2); (iv) there is a gradual decline in the numbers of resident peritoneal lymphocytes and MØ after ^{89}Sr. The resident MØ eventually reached 40-75% of the control values (Figure 3).

The data suggesting that there may be a late decrease in the resident tissue MØ are provocative, and will be pursued in additional experiments. It is interesting that this is the one situation where a decrease was observed in natural resistance to Listeria, and in the degree of enhanced protection against HSV-2 that could be produced by MVE-2 immunomodulator treatment. In another project, Dr. Yoshimi Shibata in Dr. Volkman's laboratory is investigating the question of whether there is a qualitiative change in mononuclear phagocytes produced by the bone marrow in normal mice as compared with the spleen which assumes much of the hematopoietic function after ^{89}Sr treatment.

The kinetics experiments have been complicated by two problems: (i) the presence of murine hepatitis virus (MHV) infection in some experiments, and (ii) our growing realization of the importance of the specific activity of the ^{89}Sr preparations in relation to the degree and duration of bone marrow suppression. These complications, however, have provided additional information that is proving to be useful in our overall characterization of the effects of ^{89}Sr on nonspecific effector cell populations.

While the issue of MHV infection has been a complication in the kinetics studies, it has posed an even more serious problem in interpretation of experiments with immunomodulators. Inapparent infection with MHV can increase the natural resistance to EMC virus to such an extent that enhancement of resistance by immunomodulators may be masked (Table 2). The infections (encountered both at ECU and MCP) hampered our progression with the ^{89}Sr experiments. We have taken the following steps to alleviate the problem: (i) We have worked closely with Charles River veterinary staff to minimize infection of mice during the transit process. On at least two occasions, some mice seroconverted in such a short time after arrival that infection in transit was considered probable. We have also explored the use of other sources of CD1 mice; we have not changed sources yet because of our extensive background data with the Charles River barrier raised CD1 mouse in the ^{89}Sr system; (ii) We perform routine serology on selected mice in each shipment. These are sent to independent laboratories for Elisa testing for seroconversion to MHV. The results, however, are retrospective rather than prophylactic for the problem!; (iii) We have instituted rigorous animal handling procedures to minimize cross infection from other mice to the ^{89}Sr mice, including the use of laminar flow barrier modules, filter tops, separate areas for our mice, and periodic complete disinfection of the animal rooms. This has helped, but is not a complete answer; (iv) We are converting to the use of the micro-isolator program developed by Robert Sedlacek, and renovating our animal area for an isolation unit for immunological work with mice.

We view the resolution of the inapparent infection problem as paramount in developing uniform testing for immunomodulator efficacy.

The other issue that has assumed importance is the variation in specific activity of the ^{89}Sr used in experiments over the past several years. The specific activities have ranged from 36.8 uCi/mg to 192 uCi/mg Sr for preparations purchased from Amersham. ^{89}Sr purchased from Oak Ridge, on the other hand, is nearly carrier-free and the approximate SA of their product is usually 6 Ci/mg Sr. Although the ^{89}Sr literature does not deal with the influence of SA on biological effects, we have recently noted important differences. When high SA preparations are employed, the depletion of bone

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marrow macrophage colony-forming cells is longer lasting and blood monocyte depression more sustained than when the lower SA are used. Other parameters such as the depression of indomethacin-sensitive suppressor MØ are also more transient in mice treated with low SA ^{89}Sr.

This apparent SA dependence is not entirely a frustration since it may be possible to control the time of bone marrow regeneration by reducing the SA of a ^{89}Sr preparation. We are currently studying this possibility by adding carrier (^{88}Sr) to high SA preparations prior to administering them to mice. Ideally one would wish to limit purchases to the Oak Ridge product. However, the Oak Ridge product is not consistently available, and contamination with unacceptably high levels of ^{90}Sr is often a problem.

2.2 Effects of short term and long term ^{89}Sr on immunomodulators.

Preliminary experiments have been performed to determine the effects of immunomodulators on various effector cell populations. These have been performed simultaneously with the use of the immunomodulators in protection experiments.

We have previously reported on experiments in the early period after ^{89}Sr, when C. parvum was inoculated on 3 days after ^{89}Sr and cell populations analyzed 6-7 days later (9-10 days after ^{89}Sr). C. parvum caused exudation of PMN into the peritoneal cavity, but there was no apparent exudation of monocytes. The peritoneal MØ, however, exhibited antitumor activity and showed the usual ectoenzyme phenotype associated with C. parvum treatment in both control or ^{89}Sr treated mice. The data obtained on NK cell activity shows that 7 days after C. parvum there is a marked reduction in both spontaneous and interferon- inducible NK cell activity in control mice. In the long term ^{89}Sr treated mice, NK activity was already low and C. parvum appeared to reduce this activity even further.

One experiment has been performed to measure the effects of MVE-2 on various cells at 1 and 7 days after treatment with this immunomodulator in mice treated with ^{89}Sr 8 days earlier (Table 3,4). In control mice, MVE-2 caused in one day an acute inflammatory response characterized by a profound increase in exudate PMN and a decrease in MØ. In the ^{89}Sr treated mice, MVE-2 did not cause this marked PMN exudate, but did decrease the peritoneal MØ. This may be associated with the margination of peritoneal MØ to peritoneal membranes. At one day after MVE-2, there was no increase in splenic NK cell activity in either control or ^{89}Sr treated mice. ^{89}Sr treatment itself caused the usual reduction in NK cell activity, and this apparently was not reversed by MVE-2 treatment.

By 7 days after MVE-2 treatment in control mice, the immunomodulator caused an increase in circulating monocytes and PMN in both control and ^{89}Sr treated mice. The peritoneal MØ had returned toward normal levels, and the increase in PMN in the peritoneal cavity persisted. In the ^{89}Sr treated mice, there was much less of the PMN exudate. Treatment with MVE-2 produced the expected ectoenzyme phenotype in peritoneal MØ in both control and ^{89}Sr treated mice. There was a marked reduction in 5' -nucleotidase and alkaline

phosphodiesterase with an increase in leucine aminopeptidase. These data indicate that MVE-2, like C. parvum and thioglycollate broth, can activate resident peritoneal MØ in situ.

The different effects on PMN exudation in these experiments with C. parvum and MVE-2 are worth noting. In the MVE-2 experiments, MVE-2 was not administered until 8 days after ^{89}Sr treatment, at a time when we have documented that circulating PMN are markedly decreased. Under these conditions, it appears that acute inflammatory influx of PMN may be diminished. In our previous and present experiments with C. parvum, the immunomodulator was administered on day 3 after ^{89}Sr. This may be too soon to have sufficiently decreased circulating PMN to cause a decrease in PMN exudation. Additional experiments will be performed to probe these issues. Our previous data (Volkman et al., Lab. Invest. 49:291,1983) indicate that a significant proportion of PMN may remain at 3-5 days after ^{89}Sr, while monocytes are already markedly decreased.

2.3 Effect of short term and long term ^{89}Sr on natural resistance to Listeria, EMC and HSV-2.

One to two experiments have now been performed in each of the experimental systems, as part of experiments measuring the effects of ^{89}Sr on immunomodulator enhancement of resistance. In general, there has been little change between the LD_{50} for these microorganisms in normal mice and in mice treated with ^{89}Sr (Table 5). The one exception has been our only experiment with Listeria in mice treated with the long term ^{89}Sr regimen; natural resistance was decreased 93 fold.

Our preliminary conclusions from these data are that:

(i) Natural resistance to EMC and HSV-2 infections in CD1 mice does not depend upon normally functioning circulating monocytes and NK cells, but may rely on intact tissue MØ. Future experiments will be directed toward establishing these conclusions further by showing that NK cells are not increased by the virus infections in ^{89}Sr treated mice, and that there is not elicitation of inflammatory foci in target organs. Preliminary histopathology has shown mild sporadic inflammation in control mice infected with EMC virus, and no apparent inflammation in ^{89}Sr mice infected with EMC virus.

(ii) Natural resistance to Listeria does not depend upon normally functioning circulating monocytes and NK cells. However, there is a cell population that is affected by long term exposure of mice to ^{89}Sr that may be important for natural resistance to Listeria. It is provocative to speculate that the decline in resistance may involve the gradual decline in resident peritoneal MØ. We plan to confirm the decrease in natural resistance to Listeria, and to define some of the cellular changes that may be involved.

(iii) An incidental finding has been that there is no marked change in natural resistance of the CD1 mouse to murine hepatitis virus infection in either the short term or long term ^{89}Sr regimen. We have documented early widespread MHV infection by seroconversion in two experiments, one at MCP

and one at ECU_{89} These data indicate that the MHV humoral immune response was intact in the ^{89}Sr animal, even in the face of decreased lymphocyte numbers. There was no change in the clinical picture of the mice, and no increased mortality. Mice taken from groups that show widespread seroconversion, however, tend to have the following characteristics: increased numbers of peritoneal cells, changes in the ectoenzyme phenotype toward the pattern associated with immunomodulator treatment, increased spontaneous NK cell activity that can not be increased further by exogenous interferon treatment in vitro, and increased natural resistance to EMC infection (Table 2). These changes were seen in both control and ^{89}Sr treated mice.

3.4 Effect of short term and long term ^{89}Sr on immunomodulator enhanced resistance.

For the sake of brevity, Table 6 summarizes the results of evaluating selected immunomodulators at various regimens for ability to enhance resistance of normal CD1 mice to Listeria, EMC and HSV-2. The detailed data are available on request.

Considerable investigation has been performed with therapeutic interferon treatment against EMC virus infection (Table 7). Clearly, therapeutic treat- with either mouse beta interferon or human alpha A/D recombinant interferon can be protective as measured by increased numbers of surviving mice and increased survival times. The efficacy of the interferon is critically dependent upon the amount of interferon administered and the microbial challenge level. The greatest protection was seen with treatment with at least 10,000 units of interferon and a challenge of less than 5 LD_{50} doses of EMC virus. In the three experments where the beta and alpha interferons have been compared, the alpha interferon appears to be more effective. In the coming year, we hope to be able to evaluate gamma interferon in a similar comparative manner.

Several experiments have been performed evaluating the efficacy in ^{89}Sr treated mice of the immunomodulator regimens shown above to be protective in normal CD1 mice. The results are summarized in Table 8. Additional data in Table 9 show that the short term regimen with ^{89}Sr does not affect C. parvum induced resistance to Listeria monocytogenes. These results are similar to those previously demonstrated in the long term ^{89}Sr system. Thus, C. parvum appears to be equally effective in normal mice, and in those treated with the short term and long term regimens of ^{89}Sr. These data point to C. parvum activation of tissue MØ in target organs (peritoneal cavity, liver, spleen) as being important in the enhanced resistance to this bacterial infection, which is manifest within the first 48 hours after infection.

Three experiments have been performed evaluating the efficacy of the polyanion MVE-2 and the lipoidal amine CP20,961 (Avridine) against HSV-2 infection in normal mice, and in mice treated with the short term or long term ^{89}Sr regimens. Treatment with the immunomodulator, CP20,961 in liposomes, was equally protective in normal mice and in mice early after ^{89}Sr (Table 10). Prophylactic treatment with MVE-2 one day prior to infection was also equally protective in normal mice and in mice early after ^{89}Sr treatment (Table 11). Resistance was increased over 100 fold.

Interestingly, this increased resistance did not appear to be associated with enhanced NK cell activity; spontaneous or interferon-inducible NK cell activity was low in the ^{89}Sr treated mice and was not altered by MVE-2 treatment. Additional kinetics of NK cell activity will be required to establish further this conclusion. Treatment with MVE-2 was also protective against HSV-2 in mice treated with the long term regimen with ^{89}Sr, but it did not appear to be quantitatively as protective as in normal mice (Table 12).

We have previously shown that prophylactic treatment with C. parvum was protective against EMC virus infection in normal mice and in mice early after ^{89}Sr treatment. Similar treatment with C. parvum in normal and long term ^{89}Sr treated mice also was protective (Table 13). This experiment was hampered by the increased natural resistance that was produced by inapparent MHV infection, but the data still demonstrate enhanced protection associated with C. parvum treatment.

We have also performed two experiments using interferon treatment against EMC virus infection in normal mice and in mice early after ^{89}Sr. The first experiment was hampered by inapparent MHV infection. That viral infection increased the natural resistance of mice markedly, presumably through interferon, so that little additional benefit was observed with the exogenous interferon treatment. The second experiment was hampered by having too high a challenge level of EMC virus in the interferon treatment groups. Statistical analysis of the data from the two experiments indicated that there was a small, but significant, increase in resistance with the interferons in both control and ^{89}Sr treated mice (Table 14). Quantitative comparisons of efficacy, however, were not able to be obtained. Thus, these experiments were very disappointing. Each involved considerable expense associated both with the ^{89}Sr and the use of 10^7 units of interferon. We do not plan to repeat such experiments until the problem with MHV infection has been completely resolved.

3.0 Publications/presentations related to this project

Volkman, A., N.C. Chang, P. Strausbach and P.S. Morahan. Differential effect of chronic monocyte deprivation on macrophage populations. Lab. Inves. 49: 291-298, 1983

Connor, J., W.L. Dempsey, G.E. Ericsson, M. Ackermann, P. Hwu and P.S. Morahan. Effects of ^{89}Sr destruction of bone marrow on mononuclear phagocytes. Philadelphia Immunology Meeting, October 1983.

Morahan, P.S., J. Connor, G.E. Ericsson, W.L. Dempsey, A. Volkman and P. Hwu. Effects of ^{89}Sr destruction of bone marrow on mononuclear phagocytes. Fed. Proc., June 1984.

Morahan, P.C., W.L. Dempsey and A. Volkman. Use of ^{89}Sr to define the role of tissue macrophages, circulating monocytes and NK cells in natural and immunomodulator induced antiviral resistance. Symposium on Pathobiology and Immunopathology of Virus Infections, Sendai, Japan, September 1984.

Morahan, P.S. and A. Volkman. Effects of bone marrow destruction with ^{89}Sr on natural resistance to viruses and resistance induced by immunomodulators. Sixth International Congress of Virology, Sendai, Japan, September 1984.

RESEARCH PLAN

For the next two year period, we propose to establish further the involvement of tissue MØ in comparison with circulating monocytes and PMN and NK cells in natural and immunomodulator enhanced resistance to microbial infections.

1.0 Continue to evaluate the efficacy of selected immunomodulators in our battery of model microbial infections which include Listeria, EMC and HSV-2 viruses. This aspect of the work is a straight forward continuation of our present experiments.

2.0 Define the effects of immunomodulators on various nonspecific cell populations in the ^{89}Sr systems.

3.0 Develop effective methods to decrease tissue MØ in normal and ^{89}Sr treated mice.

FIG. 1

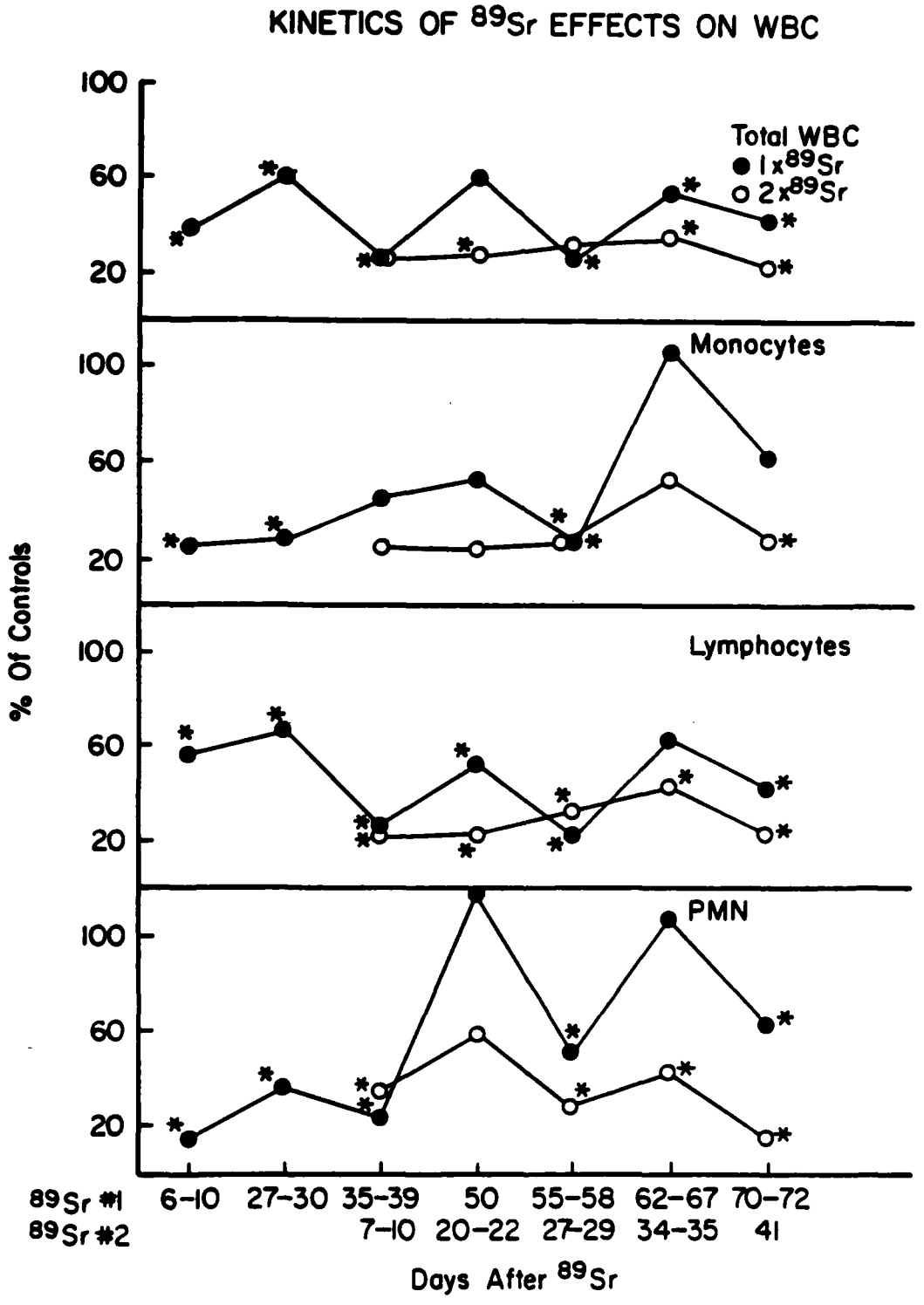

KINETICS OF 89Sr EFFECTS ON WBC
Total WBC
● 1x89Sr
○ 2x89Sr
Monocytes
Lymphocytes
PMN
% Of Controls
100
60
20
89Sr #1 6-10 27-30 35-39 50 55-58 62-67 70-72
89Sr #2 7-10 20-22 27-29 34-35 41
Days After 89Sr

FIG 2

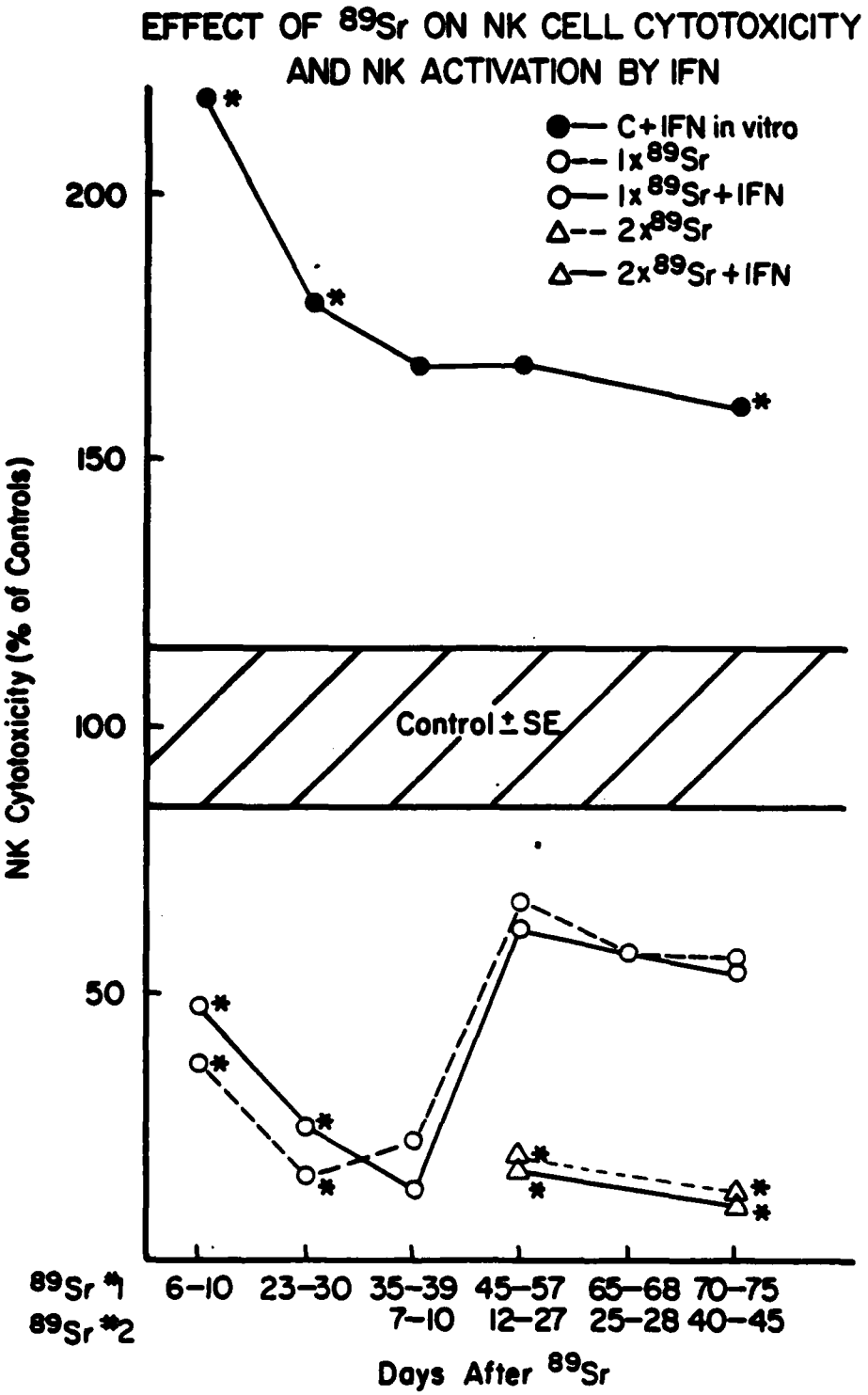

EFFECT OF 89Sr ON NK CELL CYTOTOXICITY
AND NK ACTIVATION BY IFN
C+IFN in vitro
1x89Sr
1x89Sr+IFN
2x89Sr
2x89Sr+IFN
200
150
100
50
Control ± SE
NK Cytotoxicity (% of Controls)
89Sr #1 6-10 23-30 35-39 45-57 65-68 70-75
89Sr #2 7-10 12-27 25-28 40-45
Days After 89Sr

FIG. 3

KINETICS OF ^{89}Sr EFFECTS ON PERITONEAL CELLS

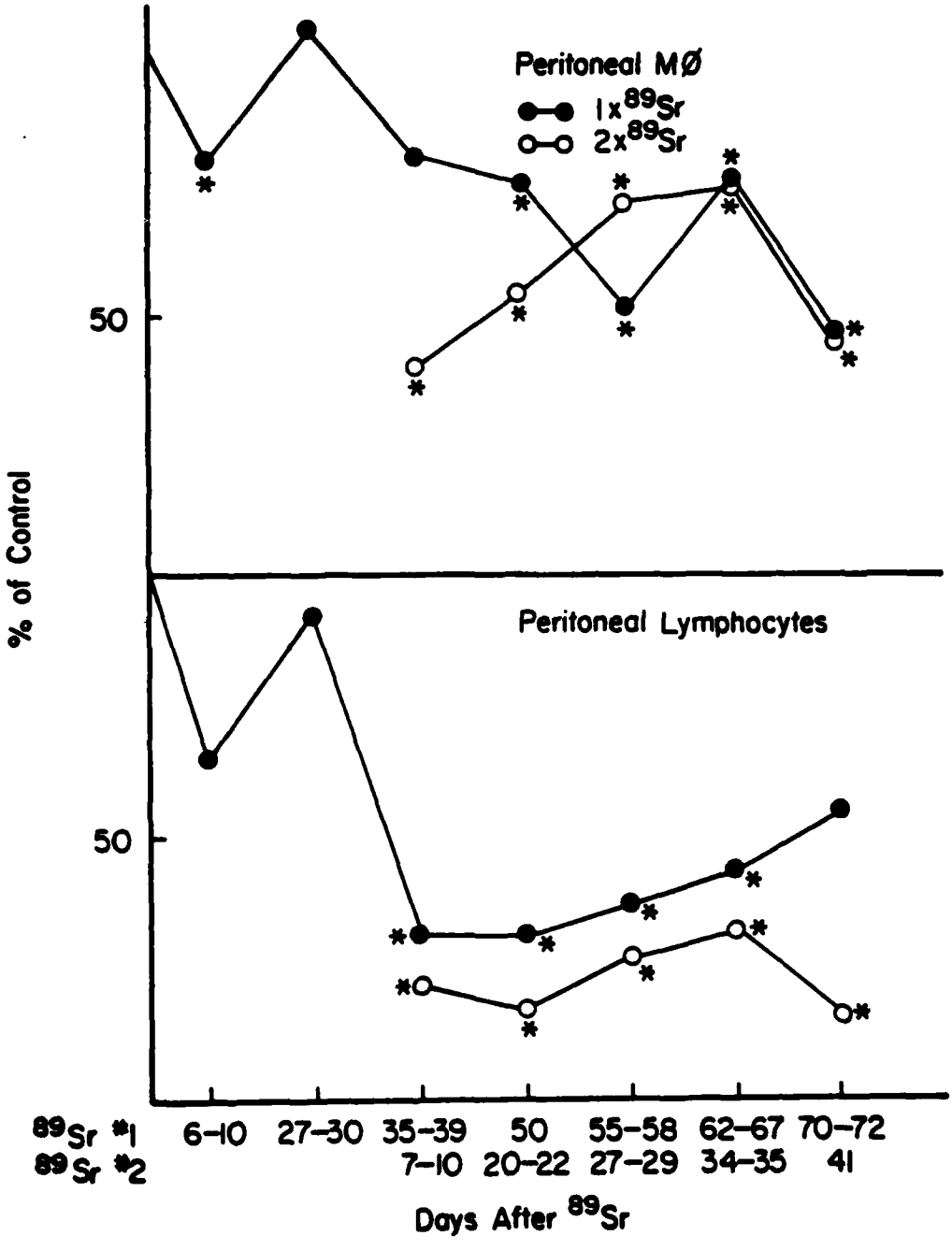


TABLE 1

Effect of ^{89}Sr on Elicitation of Peritoneal Exudate Cells by Thioglycollate Broth[a]

Treatment (days)			PEC X 10^6/mouse		Ectoenzyme SA (n mol/mg protein/min)		
^{89}Sr	^{89}Sr	TG	Total	MØ	5'N	APD	LAP
Exp 1 (SA = 76 uCi/mg)							
-	-	-	6.9±1.0	5.0±0.6	33.7±2.2	26.8±0.8	5.2±0.2
-	-	-4	14.6±1.6 (212%)	11.0±1.4 (220%)	0 (1%)	29.4±0.6	19.6±1.2 (377%)
-9	-	-	5.0±0.3	4.0±0.3	24.5±3.0	27.0±0.9	5.4±0.4
-9	-	-4	7.0±1.3	5.2±1.0	0.3±0.1 (1%)	30.9±0.6	29.5±2.6 (546%)
Exp 2 (SA = 73 and 69 uCi/mg)							
-	-	-	9.0±1.2	6.4±0.9	26.2±1.7	27.2±0.2	6.2±0.4
-	-	-5	15.1±2.4 (170%)	8.9±1.6	0.6±0.2 (2%)	33.5±0.4	40.2±1.9 (648%)
-57	-29	-	3.0±0.3[b]	2.4±0.2[b]	18.8±1.6	30.4±0.8	5.8±0.6
-57	-29	-5	2.5±0.4	1.9±0.4	1.9±0.6 (10%)	19.6±3.2	26.2 (451%)
-57	-	-	4.3±0.4[b]	3.2±0.3[b]	19.7±1.9	24.4±0.7	6.9±0.6
-57	-	-5	10.6±3.2 (246%)	8.1±2.8 (253%)	1.0±0.2 (5%)	30.3±0.4	39.3±2.9 (570%)
Exp 3 (SA = 66 and 59 uCi/mg							
-	-	-	10.1±1.0	6.8±0.7	44.0	13.9±1.2	13.5±0.9
-	-	-5	24.9±1.2 (246%)	16.5±1.6 (243%)	1.4 (3%)	37.2 (268%)	25.8 (191%)
-63	-35	-	6.8±0.7[b]	4.9±0.5	27.0	15.9±3.2	3.4±0.4[b]
-63	-35	-5	17.6±2.0 (259%)	11.3±1.8 (231%)	1.9 (7%)	40.8 (257%)	37.1 (1090%)
-	-	-	7.8±0.4	4.0±0.7	34.0	16.6±0.6	4.0±0.3
-67	-	-	6.2±0.7	4.8±0.3	33.4	14.7	2.0
-67	-	-5	18.3±2.4 (295%)	ND	2.0 (6%)	41.1 (280%)	36.1 (1805%)
Exp 4 (SA = ca. 60,000 uCi/mg)							
-	-	-	2.4±0.5	1.0±0.2	ND	ND	ND
-	-	-5	11.2±1.0 (467%)	8.0±0.7 (800%)			
-30	-	-	2.0±0.5	1.0±0.2			
-30	-	-5	3.5±1.6	2.0±0.6			
-	-	-	3.2±0.7	1.3±0.2	ND	ND	ND
-	-	-5	11.0±4.9 (344%)	7.6±3.3 (585%)			
-58	-	-	1.5±0.3	0.7±0.1			
-58	-	-5	5.9±0.7 (393%)	4.2±2.0 (600%)			
-	-	-	3.4±0.1	1.7±0.3	ND	ND	ND
-	-	-5	12.4±1.8 (365%)	8.8±1.7 (518%)			
-63	-	-	2.2±0.4	1.0±1.1			
-63	-	-5	7.4±1.1 (336%)	5.0±0.7 (500%)			

[a] Mice were inoculated i.p. (Exps 1-3) or i.v. (Exp 4) with 4 uCi/gbw ^{89}Sr and elicitation of cells with Brewer's Thioglycollate broth CTG measured of the times indicated. Significant changes due to the thioglycollate inflammatory stimulus are shown in parenthesis.

[b] $p < 0.05$ for the ^{89}Sr group in comparison with the control group.

TABLE 2

Effect of Inapparent MHV Infection on Natural Resistance to EMC Virus

$\log_{10}$ virus dilution	Exp. No. / Date	Dead/Total in 1 Jan 83	2 Mar 83	3 Aug 83	4 Sept 83	5 Dec 83	6 Jan 84
-5		ND	ND	ND	7/7	ND	8/8
-6		8/9	ND	2/8	6/7	7/8	8/8
-6.5		8/8	8/8	ND	7/7	6/8	ND
-7		6/8	13/16	1/8	7/8	3/8	8/8
-7.5		6/8	5/8	ND	4/8	1/8	ND
-8		2/8	0/8	0/8	2/7	0/8	8/8
$\log_{10}$ LD_{50}		7.7	7.5	6.0	7.6	6.8	8.0
Evidence of MHV		No	No	Yes	No	Yes	No

TABLE 3

Effect of Short-term ^{89}Sr on Immunomodulatory Effects of MVE-2 at One Day - Preliminary Data

Treatment ^{89}Sr	Treatment MVE-2	WBC X 10^5/ml +SE Total	Mono	L	PMN	PC X 10^6/mouse +SE Total	MØ	PMN	Splenic NK +SE - IFN	+ IFN
-	-	35.1±2.5	0.7±0.2	28.3±3.0	5.2±1.3	6.9±1.0	5.0±0.6	0.1±0.04	6.1±1.7	20.4±4.3
-	+	31.0±4.6	0.7±0.1	16.9±3.3	13.2±1.6	5.4±0.9	1.8±0.5 (36%)	2.3±0.6 (2300%)	10.2	10.4
+	-	15.5±1.2	0.6±0.2	13.6±0.8	2.0±0.6	5.0±0.3	4.0±0.3	0.2±0.1	2.8±0.7[b]	6.2±1.8[b]
+	+	12.6±1.5	0.2±0.05	9.6±0.7	2.9±1.1	2.0±0.2	1.1±0.1 (28%)	0.4±0.05 (200%)	2.2	4.2

[a] CD1 female mice were inoculated i.p. on day 0 with saline or ^{89}Sr (4 uCi/gbw, SA = 78 uCi/mg), inoculated i.p. on day 8 with 50 mg/kg MVE-2, and cell populations analyzed twenty-four hours later. Marked changes associated with MVE-2 treatment are indicated by the percentages in parentheses.
[b] $p<0.05$ of ^{89}Sr as compared with the control group.

TABLE 4

Effect of Short-term ^{89}Sr on Immunomodulating Effects of MVE-2 at seven days - preliminary data

Treatment ^{89}Sr	MVE-2	Spleen Weight (mg)	WBC X 10^5/ml Total	Mono	L	PMN	PC X 10^6/mouse Total	MØ	PMN	MØ Ectoenzyme SA (nmols/mg prot/min) 5'N	APD	LAP
-	-	79.5±5.4	35.2±4.8	1.6±0.5	22.2±3.5	10.5±2.1	7.5±0.8	5.6±0.6	0.1±0.02	30.7±2.4	28.9±1.7	5.1±0.6
-	+	235.5±26.8 (297%)	57.2±9.3 (162%)	8.0±2.7 (500%)	15.0±2.1	34.0±6.1 (323%)	4.1±0.4	2.2±0.2	1.0±0.2 (1000%)	0.6±0.5 (2%)	8.9±0.5 (31%)	20.4±0.7 (400%)
+	-	111.3±16.4	11.5±1.7	0.7±0.2	7.0±1.1	3.8±0.8	4.8±0.5	3.8±0.5	0.2±0.1	35.2±3.0	13.4±0.6	5.0±0.2
+	+	262.2±20.5 (236%)	23.2±5.3 (201%)	4.8±1.0 (685%)	6.1±2.1	12.2±4.2 (321%)	5.9±0.6	4.3±0.5	0.5±0.1 (250%)	0.1±0.1 (1%)	9.6±0.7 (72%)	16.7±1.2 (334%)

[a]CD-1 female mice were inoculated on day 0 i.p. with saline or ^{89}Sr (4 uCi/gbw, SA = 78 uCi/mg), inoculated i.p. on day 8 with 50 mg/kg MVE-2 and cell populations analyzed 7 days later (15 days after ^{89}Sr). Marked changes associated with MVE-2 treatment are indicated by the percentages in parentheses.

TABLE 5

Summary of Effects of Short-term and Long-term ^{89}Sr on Natural Resistance of CD1 female mice

	^{89}Sr regimen	
	Short-term	Long-Term
HSV-2	No significant changes (2 exp)	No significant changes (1 exp)
EMC	No significant changes (2 exp)	No significant changes (1 exp)
Listeria monocytogenes	No significant changes (1 exp)	Significant decrease, 93 fold (1 exp)

In some experiments with no significant change, a 2-5 fold decrease in LD_{50} has been observed.

TABLE 8

Summary - Effectiveness of Immunomodulators in Increasing Resistance in ^{89}Sr Treated Mice

	Treatment			Effect in ^{89}Sr as Compared with Control Mice	
Microorganism	Drug	Dose	Schedule	Short-term	Long-term
EMC Virus	C. parvum	35mg/kg	D-7	Effective	Effective
	a A/D IFN	10,000IU	D 0 to 6	Effective	
	B IFN	10,000IU	D 0 to 6	Effective	
HSV-2	MVE-2	50mg/kg	D-1	Effective	Effective*
	CP20,961	50mg/kg	D-1	Effective	
Listeria monocytogenes	C. parvum	35mg/kg	D-7	Effective	Effective

*The degree of protection (change in LD_{50} between control and drug - treated mice) was not as great as in normal mice.

TABLE 6

Summary of Protective Effects of Immunomodulators in Normal CD1 Mice

Treatment			Effects Against Infection With		
Drug	Dose	Schedule	HSV-2	EMC	Listeria
C. parvum	35mg/kg	D-7	+	+	+
"	"	D-1	+	+	NC
Pyran	50mg/kg	D-7			+
"	"	D-1	+	+	-
MVE-2	50mg/kg	D-7			+
"	"	D-1	+	+	NC
CP20,961 in Liposomes	50mg/kg	D-7			-
	"	D-1	+	+	-
β IFN	10,000 IU	BID, D 0 to6	+	+	
α A/D IFN	10,000 IU	BID, D 0 to6		+	
N-acetyl muramyl-L-alanyl-D-isoglutamine	8mg/kg	D-7			NC
	"	D-1			NC
"	4mg/kg	D-7			-
"	"	D-1			NC
N-acetyl muramyl-D-alanyl-D-isolglutamine	4mg/kg	D-7			-

+ = Increased resistance shown by significantly decreased mortality and/or increased survival time.

- = Decreased resistance shown by significantly increased mortality and/or decreased survival time.

NC = No significant change in resistance.

TABLE 7

Summary of Effects of β IFN on EMC Infection in Normal CD1 Mice

Treatment IFN	Dose (IU)	Schedule Days	Mortality Dead/Total (%)[a]		Survival Analysis Median Survival Time (Days)[b]
Exp. A (5 LD_{50})					
-	-	-	13/15	(87%)	5.0
Mock IFN	-	0,1,2,3,4,5,6	15/15	(100%)	5.0
β IFN	20,000	0,1,2,3,4,5,6	0/12	(0%)[c]	>14.0[c]
Exp. B (3 LD_{50})					
-	-	-	13/16	(81%)	6
Mock IFN	-	0,1,2,3	11/12	(92%)	4
β IFN	10,000	0,1,2,3	7/12	(58%)	7.5[c]
Exp. C (0.7-6 LD_{50})					
Mock IFN			16/24	(67%)	4.0
β IFN	10,000	0,1,2,3,4,5,6	6/12	(50%)	13.5
α A/D IFN	10,000	0,1,2,3,4,5,6	6/18	(33%)[c]	>14.0[c]
Exp. D (≥5 LD_{50})					
-	-	-	7/10	(70%)	5.8
Mock IFN	-	0,1,2,3,4,5,6	10/10	(100%)	4.8
β IFN	500	0,1,2,3,4,5,6	8/10	(80%)	4.2
Exp. E (40 LD_{50})					
-	-	-	7/7	(100%)	4.0
Mock IFN	-	-	12/12	(100%)	5.0
β IFN	8,000	0,1,2,3,4,5,6	12/12	(100%)	5.0
α A/D IFN	13,000	0,1,2,3,4,5,6	5/12	(42%)	>14.0[c]
Exp. F (>1000 LD_{50})					
Mock IF			8/8	(100%)	4.0
β IFN	10,000	0,1,2,3,4,5,6	6/6	(100%)	4.0
α A/D IFN	10,000	0,1,2,3,4,5,6	6/6	(100%)	5.0

[a] Mortality analyzed by Chi square analysis
[b] Survival time analyzed by Mann Whitney U test. Days is the median survival time.
[c] $p < 0.05$

TABLE 9

Effect of Short-term ^{89}Sr on Natural and C. parvum Induced Resistance to Listeria

^{89}Sr Treatment	C. parvum	Log_{10} CFU per LD_{50} (Δlog_{10})	Log_{10} CFU±SE per spleen at 48 hr after infection (Δlog_{10})	Spleen Weight (mg ± SE)
-	-	3.9	6.6±0.4	144.3±13.2
-	+	> 5.6 (1.7)	2.1±0.2 (4.5)	443.3±3.8
+	-	3.3	7.1±0.1	138.8±7.4
+	+	5.1 (1.8)	3.2±0.7 (3.9)	610.8±94.4

CD1 female mice were inoculated i.v. with 2 uCi/gbw ^{89}Sr (SA = Ca. 60,000 uCi/mg), and were treated i.p. with 35 mg/kg C. parvum on day 3. Seven days later they were challenged i.p. with dilutions of Listeria monocytogenes. Two days later four mice from each group challenged with 1.2 X 10^5 CFU were sacrificed and the growth of Listeria in the spleen and spleen weight was measured.

TABLE 10

Effect of Short Term ^{89}Sr Treatment on Natural or Immunomodulator Induced Resistance to HSV-2 Infection in CD1 mice[a]

^{89}Sr Treatment	Agent	Number of Dead/Total (%)		Median Survival Time (Days)	Spontaneous NK (% Cytotoxicity ± SE)[d]
-	-	8/17	(47%)	17	19.9 ± 2.5
-	MVE-2	2/12	(17%)	>21	
-	CP 20,961 Liposomes	0/12	(0%)[b]	>21[c]	
+	-	11/16	(69%)	11	4.9 ± 1.9
+	MVE-2	2/12	(17%)[b]	> 21[c]	
+	CP 20,961 Liposomes	4/12	(33%)[b]	> 21[c]	

[a] CD-1 female mice were inoculated i.p. with saline or 4 uCi/gbw of ^{89}Sr (SA = 113 uCi/mg). Thirteen days later they were treated i.p. with 50 mg/kg of either MVE-2 or a CP 20,961 liposome preparation. Twenty-four hours later the mice were challenged i.p. with ca. 24,000 PFU of HSV-2, and mortality followed for 21 days.

[b] $p < 0.05$ by Chi square test

[c] $p < 0.05$ by Mann-Whitney U test

[d] Spleens were removed from mice 6 days after ^{89}Sr treatment, and NK cell activity against Yac-1 cells was measured at a 100:1 effector:target cell ratio in a 4 hour chromium release assay.

TABLE 11

Effect of Short Term ^{89}Sr Treatment on Natural Resistance to HSV-2, Protection Produced by MVE-2, and Natural Killer Cell Activity

Treatment		$\log_{10}$ PFU per LD_{50} ($\Delta \log_{10}$)	Protection after challenge with ca. 52,000 PFU		% NK Cytotoxicity[d]	
^{89}Sr	MVE-2		No. Dead/ Total (%)	Median survival Time (days)	- IFN	+ IFN
-	-	3.2	14/18 (78%)	10	6.1	20.4
-	+	5.4 (2.2)	1/18 (6%)[b]	>21[c]	10.2	10.4
+	-	2.5	17/17 (100%)	8.5	2.8	6.2
+	+	5.4 (2.9)	2/9 (22%)[b]	>21[c]	2.2	4.2

[a] CD1 female mice were inoculated i.p. with saline or 4 uCi/gbw of ^{89}Sr (SA = 76 uCi/mg), inoculated i.p. eight days later with 50 mg/kg MVE-2 or saline, challenged i.p. 24 hr later with serial dilutions of HSV-2, and mortality followed for 21 days.

[b] $p < 0.05$ by Chi square test

[c] $p < 0.05$ by Mann Whitney U test

[d] The mean of NK cell activity that was measured in spleens from two individual mice each on day 7 and day 9 after ^{89}Sr treatment, using a 100:1 effector: target cell ratio against Yac-1 cells in a 4 hr chromium release assay with and without the presence of exogenous (IFN). Activity in two mice treated with MVE-2 was measured on day 9 (one day after MVE-2 treatment).

TABLE 12

Effects of Long-term ^{89}Sr on Resistance to HSV-2 and Protection Produced by MVE-2 Immunomodulator[a]

	Log_{10} PFU/LD_{50}	Δ log_{10}
C	2.9	
C + MVE-2	5.2	2.3
^{89}Sr	2.5	
^{89}Sr + MVE-2	3.8	1.3

[a]CD1 female mice were inoculated i.p. with 4 uCi/gbw of ^{89}Sr on day 0 (SA = 73 uCi/mg) and on day 33 (SA = 69 uCi/gbw), were inoculated i.p. on day 64 with 50 mg/kg MVE-2, challenged i.p. 24 hr later with dilutions of HSV-2, and mortality followed for 21 days.

TABLE 13

Effect of Long-term ^{89}Sr Treatment on Natural and C. parvum Induced Resistance to EMC Virus

Treatment Schedule (days)		Dead/Total at Virus Dilutions of				
^{89}Sr	C. parvum	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
-	-	ND	ND	2/8 (25%)	1/8 (12%)	0/8 (0%)
-	+	4/8 (50%)	0/8 (25%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
+	-	ND	ND	3/8 (38%)	0/8 (0%)	0/8 (0%)
+	+	3/8 (38%)	3/8 (38%)	0/8 (0%)	0/8 (0%)	ND

CD1 female mice were inoculated i.v. with 4 uCi/gbw of ^{89}Sr on day - 62 (SA = 67uCi/mg) and on day - 34 (SA = 94 uCi/mg). On day - 7 they reviewed C. parvum i.p. (35 mg/kg) and were challenged i.p. on day 0 with dilutions of EMC virus.

TABLE 14

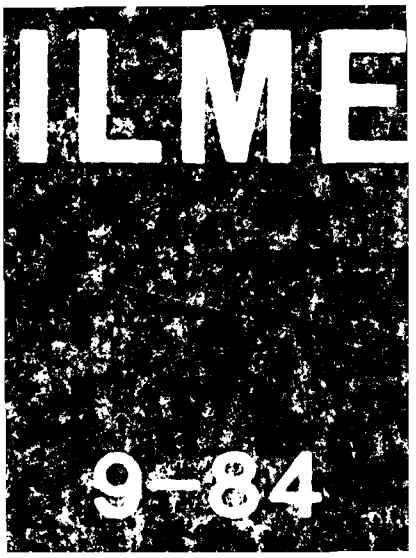
Effect of Short Term ^{89}Sr on Natural and Interferon Induced Resistance to EMC Virus

Groups		Experiment 1				Experiment 2	
		Overall Survival Analysis[c]		Mortality and Survival at 0.7-6LD[b]		Overall Survival Analysis[c]	
^{89}Sr	IFN	$\log_{10}$ LD_{50}	Survival Dist. (p value)	# Dead/Total (%)	Survival Time ± SE (p value)	$\log_{10}$ LD_{50}	Survival Dist. (p value)
-	-	6.8		16/24 (67%)	3.7 ± 0.3	8.0	
-	a A/D	6.5	0.01	6/12 (50%)	7.5 ± 2.0 (0.08)	5.0	0.13
-	β	6.2	0.05	6/18 (33%)	5.2 ± 1.2 (0.02)	5.0	0.43
+	-	7.0		10/16 (62%)	4.5 ± 0.5	8.0	
+	a A/D	6.2	0.04	4/12 (33%)	6.5 ± 2.0 (0.13)	6.0	0.04
+	B	6.7	0.16	4/12 (33%)	4.5 ± 2.0 (0.24)	6.0	0.06

[a] CD1 female mice were inoculated i.p. with 4 uCi/gbw ^{89}Sr (SA = 165 uCi/mg and challenged i.p. with dilutions of EMC virus 10 days later. Mice were treated i.p. with 10,000 IU of A/D human interferon or β mouse interferon beginning 2 hrs after virus infection and continuing twice a day for six days.

[b] Survival distribution of the two combined groups challenged with 0.7 - $6LD_{50}$ was analyzed by the BMDP 1L life tables. Survival indicates the time estimated when 75% of the mice were surviving.

[c] The overall survival of all groups inoculated with the various dilutions of EMC was measured by LD_{50} calculated by Reed-Meunch procedure, and survival distribution times analyzed by Cox's proportional hazards general linear model.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